# IN THE UNITED STATES PATENT AND TRADEMARK OFFICE



In re PATENT APPLICATION of
Inventor(s): CRESSWELL et al.

| Appln. No.: | to be | assigned |
|---|---|---|
| Series Code | ↑ | ↑ Serial No. |

Group Art Unit: to be assigned

Filed: August 10, 2001

Examiner: to be assigned

Title: Chemical Compounds

| Atty. Dkt. | P 0282794 | 100139/US |
|---|---|---|
| | M# | Client Ref |

Date: August 10, 2001

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Hon. Asst Commissioner of Patents
Washington, D.C. 20231

Sir:

Please accept the enclosed certified copy(ies) of the respective foreign application(s) listed below for which benefit under 35 U.S.C. 119/365 has been previously claimed in the subject application and if not is hereby claimed.

| Application No. | Country of Origin | Filed |
|---|---|---|
| 00 205 44.3 | GB | August 22, 2000 |

Respectfully submitted,

Pillsbury Winthrop LLP
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Signed

Dated 26 June 2001

**Patents Form 1/77**

Patents Act 1977
(Rule 16)

The Patent Office

1/77

THE PATENT OFFICE
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22 AUG 2000
NEWPORT

The Patent Office

Cardiff Road
Newport
Gwent NP9 1RH

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*(See the notes on the back of this form. You can also get an explanatory leaflet from the Patent Office to help you fill in this form)*

1. Your reference — 100139

2. Patent application number *(The Patent Office will fill in this part)* — **0020544.3** — 22 AUG 2000

3. Full name, address and postcode of the or of each applicant *(underline all surnames)*

AstraZeneca AB
S-151 85 Sodertalje
Sweden

22AUG00 E562439-1 002934
P01/7700 0.00-0020544.3

Patents ADP number *(if you know it)* 7822448003

If the applicant is a corporate body, give the country/state of its incorporation

4. Title of the invention — CHEMICAL COMPOUNDS

5. Name of your agent *(if you have one)* — Allen Giles 6988463003

"Address for service" in the United Kingdom to which all correspondence should be sent *(including the postcode)*

AstraZeneca UK Ltd
Global Intellectual Property Department - Patents
PO BOX 272, Mereside, Alderley Park
Macclesfield, Cheshire, SK10 4GR
England

Patents ADP number *(if you know it)*

6. If you are declaring priority from one or more earlier patent applications, give the country and the date of filing of the or of each of these earlier applications and *(if you know it)* the or each application number

| Country | Priority application number *(if you know it)* | Date of filing *(day / month / year)* |
|---|---|---|

7. If this application is divided or otherwise derived from an earlier UK application, give the number and the filing date of the earlier application

| Number of earlier application | Date of filing *(day / month / year)* |
|---|---|

8. Is a statement of inventorship and of right to grant of a patent required in support of this request? *(Answer 'Yes' if:*

*a) any applicant named in part 3 is not an inventor, or*
*b) there is an inventor who is not named as an applicant, or*
*c) any named applicant is a corporate body.*
*See note (d))*

# CHEMICAL COMPOUNDS

This invention relates to polymorphisms in the human MCT-1 gene and corresponding novel allelic polypeptides encoded thereby. The invention also relates to methods and materials for analysing allelic variation in the MCT-1 gene, and to the use of MCT-1 polymorphism in treatment of diseases with MCT-1 transportable drugs.

Monocarboxylic acids play a major role in the metabolism of all cells, with lactic acid, the end product of glycolysis, being especially important. Some tissues, such as white skeletal muscle, red blood cells and many tumour cells, rely on this pathway to produce most of their ATP under normal physiological conditions, while all tissues become dependent on this pathway during hypoxia or ischaemia. Glycolysis produces two molecules of lactic acid for every glucose molecule consumed, and these must be transported out of the cell if high rates of glycolysis are to be maintained. If efflux of lactic acid from the cell does not keep pace with production, intracellular concentrations increase and cause the pH of the cytosol to decrease. This leads to inhibition of phosphofructokinase and hence glycolysis. Other tissues, such as brain, heart and red skeletal muscle, readily oxidize lactic acid, which may become a major respiratory fuel under some conditions. In these tissues lactic acid must be rapidly transported into the cell. The same is true for tissues such as the liver, which, through the operation of the Cori cycle, utilise lactate as their dominant gluconeogenic substrate (Denton, R. M. and Halestrap, A. P. (1979) Essays Biochem. 15, 37-47: Juel, C. (1997) Physiol. Rev. 77, 321-358.) Although it is lactic acid that is both produced and utilized by metabolism, the pK of lactic acid is 3.86, which ensures that it dissociates almost entirely to the lactate anion at physiological pH. This charged species cannot cross the plasma membrane by free diffusion, but requires a specific transport mechanism, provided by proton-linked monocarboxylate transporters (MCTs). These transporters catalyse the facilitated diffusion of lactate with a proton. There is no energy input other than that provided by the concentration gradients of lactate and protons, although the latter, in the form of a pH gradient, can drive the accumulation or exclusion of the lactate anion (Poole, R. C. and Halestrap, A. P. (1993) Am. J. Physiol. 264, C761-C782.: Juel, C. (1997) Physiol. Rev. 77, 321-358).

MCT-1 is thought to be involved in the transport of of drugs involved in lipid lowering *e.g.* statins. Statins have been refered to as a first-line therapy for patients with atherosclerotic vascular diseases.

The term human includes both a human having or suspected of having a MCT-1 mediated disease and an asymptomatic human who may be tested for predisposition or susceptibility to such disease. At each position the human may be homozygous for an allele or the human may be a heterozygote.

The term single nucleotide polymorphism includes single nucleotide substitution, nucleotide insertion and nucleotide deletion which in the case of insertion and deletion includes insertion or deletion of one or more nucleotides at a position of a gene.

In one embodiment of the invention preferably the method for diagnosis described herein is one in which the single nucleotide polymorphism at position 1450 is presence of A and/or G.

In one embodiment of the invention preferably the method for diagnosis described herein is one in which the single nucleotide polymorphism at position 1482 is presence of A and/or T.

In another embodiment of the invention preferably the method for diagnosis described herein is one in which the single nucleotide polymorphism at position 2461 is the presence of A and/or G.

The method for diagnosis is preferably one in which the sequence is determined by a method selected from amplification refractory mutation system and restriction fragment length polymorphism.

The status of the individual may be determined by reference to allelic variation at any one, two or three or more positions.

The test sample of nucleic acid is conveniently a sample of blood, bronchoalveolar lavage fluid, sputum, or other body fluid or tissue obtained from an individual. It will be appreciated that the test sample may equally be a nucleic acid sequence corresponding to the sequence in the test sample, that is to say that all or a part of the region in the sample nucleic acid may firstly be amplified using any convenient technique e.g. PCR, before analysis of allelic variation.

It will be apparent to the person skilled in the art that there are a large number of analytical procedures which may be used to detect the presence or absence of variant nucleotides at one or more polymorphic positions of the invention. In general, the detection of allelic variation requires a mutation discrimination technique, optionally an amplification reaction and optionally a signal generation system. Table 1 lists a number of mutation

**Hybridisation Based**

Solid phase hybridisation: Dot blots, MASDA, Reverse dot blots, Oligonucleotide arrays (DNA Chips)

Solution phase hybridisation: Taqman™ - US-5210015 & US-5487972 (Hoffmann-La Roche), Molecular Beacons - Tyagi *et al* (1996), Nature Biotechnology, **14**, 303; WO 95/13399 (Public Health Inst., New York)

**Extension Based:** ARMS™, ALEX™ - European Patent No. EP 332435 B1 (Zeneca Limited), COPS - Gibbs *et al* (1989), Nucleic Acids Research, **17**, 2347.

**Incorporation Based:** Mini-sequencing, APEX

**Restriction Enzyme Based:** RFLP, Restriction site generating PCR

**Ligation Based:** OLA

**Other:** Invader assay

Table 2 - Signal Generation or Detection Systems

**Fluorescence:** FRET, Fluorescence quenching, Fluorescence polarisation - United Kingdom Patent No. 2228998 (Zeneca Limited)

**Other:** Chemiluminescence, Electrochemiluminescence, Raman, Radioactivity, Colorimetric, Hybridisation protection assay, Mass spectrometry

Table 3 - Further Amplification Methods

SSR, NASBA, LCR, SDA, b-DNA

Preferred mutation detection techniques include ARMS™, ALEX™, COPS, Taqman, Molecular Beacons, RFLP, and restriction site based PCR and FRET techniques.

Particularly preferred methods include ARMS™ and RFLP based methods. ARMS™ is an especially preferred method.

In a further aspect, the diagnostic methods of the invention are used to assess the pharmacogenetics of a drug transportable by MCT-1.

Assays, for example reporter-based assays, may be devised to detect whether one or more of the above polymorphisms affect transcription levels and/or message stability.

Individuals who carry particular allelic variants of the MCT-1 gene may therefore exhibit differences in their ability to regulate protein biosynthesis under different

According to another aspect of the present invention there is provided a human MCT-1 gene or its complementary strand comprising an allelic variant, preferably corresponding with one or more the positions defined herein or a fragment thereof of at least 20 bases comprising at least one allelic variant.

Fragments are at least 17 bases, more preferably at least 20 bases, more preferably at least 30 bases.

The invention further provides a nucleotide primer which can detect a polymorphism of the invention.

According to another aspect of the present invention there is provided an allele specific primer capable of detecting a MCT-1 gene polymorphism, preferably at one or more of the positions as defined herein.

An allele specific primer is used, generally together with a constant primer, in an amplification reaction such as a PCR reaction, which provides the discrimination between alleles through selective amplification of one allele at a particular sequence position e.g. as used for ARMS™ assays. The allele specific primer is preferably 17- 50 nucleotides, more preferably about 17-35 nucleotides, more preferably about 17-30 nucleotides.

An allele specific primer preferably corresponds exactly with the allele to be detected but derivatives thereof are also contemplated wherein about 6-8 of the nucleotides at the 3' terminus correspond with the allele to be detected and wherein up to 10, such as up to 8, 6, 4, 2, or 1 of the remaining nucleotides may be varied without significantly affecting the properties of the primer.

Primers may be manufactured using any convenient method of synthesis. Examples of such methods may be found in standard textbooks, for example "Protocols for Oligonucleotides and Analogues; Synthesis and Properties," Methods in Molecular Biology Series; Volume 20; Ed. Sudhir Agrawal, Humana ISBN: 0-89603-247-7; 1993; 1st Edition. If required the primer(s) may be labelled to facilitate detection.

According to another aspect of the present invention there is provided an allele-specific oligonucleotide probe capable of detecting a MCT-1 gene polymorphism, preferably at one or more of the positions defined herein.

The allele-specific oligonucleotide probe is preferably 17- 50 nucleotides, more preferably about 17-35 nucleotides, more preferably about 17-30 nucleotides.

disequilibrium at the cystathionine beta synthase (CBS) locus and the association between genetic variation at the CBS locus and plasma levels of homocysteine. *Ann Hum Genet* (1998) 62:481-90, De Stefano V, Dekou V, Nicaud V, Chasse JF, London J, Stansbie D, Humphries SE, and Gudnason V; and Variation at the von willebrand factor (vWF) gene locus is associated with plasma vWF:Ag levels: identification of three novel single nucleotide polymorphisms in the vWF gene promoter. *Blood* (1999) 93:4277-83, Keightley AM, Lam YM, Brady JN, Cameron CL, Lillicrap D).

According to another aspect of the present invention there is provided a computer readable medium comprising at least one novel sequence of the invention stored on the medium. The computer readable medium may be used, for example, in homology searching, mapping, haplotyping, genotyping or pharmacogenetic analysis.

According to another aspect of the present invention there is provided a method of treating a human in need of treatment with a drug transportable by MCT-1 in which the method comprises:

i) diagnosis of a single nucleotide polymorphism in MCT-1 gene in the human, which diagnosis preferably comprises determining the sequence of the nucleic acid at one or more of the following positions:

positions 1450, 1482 and 2461 in the sequence of the MCT-1 gene as defined by the position in SEQ ID NO: 13

and determining the status of the human by reference to polymorphism in the MCT-1 gene; and

ii) administering an effective amount of the drug.

Preferably determination of the status of the human is clinically useful. Examples of clinical usefulness include deciding which statin drug or drugs to administer and/or in deciding on the effective amount of the statin drug or drugs. Statins already approved for use in humans include atorvastatin, cerivastatin, fluvastatin, pravastatin and simvastatin. The reader is referred to the following references for further information: Drugs and Therapy Perspectives (12th May 1997), **9**: 1-6; Chong (1997) Pharmacotherapy **17**: 1157-1177; Kellick (1997) Formulary **32**: 352; Kathawala (1991) Medicinal Research Reviews, **11**: 121-146; Jahng (1995) Drugs of the Future **20**: 387-404, and Current Opinion in Lipidology, (1997), **8**, 362 - 368. Another statin drug of note is compound 3a (S-4522) in Watanabe (1997) Bioorganic and Medicinal Chemistry **5**: 437-444. The term "drug transportable by MCT-1"

Examples of various assays useful for such determination include those described in: *Antibodies: A Laboratory Manual*, Harlow and Lane (eds.), Cold Spring Harbor Laboratory Press, 1988; as well as procedures such as countercurrent immuno-electrophoresis (CIEP), radioimmunoassay, radioimmunoprecipitation, enzyme-linked immuno-sorbent assays (ELISA), dot blot assays, and sandwich assays, see U.S. Patent Nos. 4,376,110 and 4,486,530.

Monoclonal antibodies may be readily prepared using well-known procedures, see for example, the procedures described in U.S. Patent Nos. RE 32,011, 4,902,614, 4,543,439 and 4,411,993; Monoclonal Antibodies, Hybridomas: *A New Dimension in Biological Analyses,* Plenum Press, Kennett, McKearn, and Bechtol (eds.), (1980).

The monoclonal antibodies of the invention can be produced using alternative techniques, such as those described by Alting-Mees et al., "Monoclonal Antibody Expression Libraries: A Rapid Alternative to Hybridomas", *Strategies in Molecular Biology* 3: 1-9 (1990) which is incorporated herein by reference. Similarly, binding partners can be constructed using recombinant DNA techniques to incorporate the variable regions of a gene that encodes a specific binding antibody. Such a technique is described in Larrick et al., *Biotechnology*, 7: 394 (1989).

Once isolated and purified, the antibodies may be used to detect the presence of antigen in a sample using established assay protocols, see for example "A Practical Guide to ELISA" by D. M. Kemeny, Pergamon Press, Oxford, England.

According to another aspect of the invention there is provided a diagnostic kit comprising an antibody of the invention.

The invention will now be illustrated but not limited by reference to the following Examples. All temperatures are in degrees Celsius.

In the Examples below, unless otherwise stated, the following methodology and materials have been applied.

AMPLITAQ™ ,available from Perkin-Elmer Cetus, is used as the source of thermostable DNA polymerase.

General molecular biology procedures can be followed from any of the methods described in "Molecular Cloning - A Laboratory Manual" Second Edition, Sambrook, Fritsch and Maniatis (Cold Spring Harbor Laboratory, 1989).

For dye-primer sequencing these primers were modified to include M13 forward and reverse primer sequences (ABI protocol P/N 402114, Applied Biosystems) at the 5' end of the forward and reverse oligonucleotides respectively.

MCT1 oligos for cDNA Amplification

| | |
|---|---|
| Product 1F (13-32) | SEQ ID NO 1 |
| Product1R (559-578) | SEQ ID NO 2 |
| Product 2F (429-448) | SEQ ID NO 3 |
| Product 2R (948-967) | SEQ ID NO 4 |
| Product 3F (778-797) | SEQ ID NO 5 |
| Product 3R (1428-1409) | SEQ ID NO 6 |
| Product 4F (1243-1262) | SEQ ID NO 7 |
| Product 4R (1837-1818) | SEQ ID NO 8 |
| Product 5F (1667-1786) | SEQ ID NO 9 |
| Product 5R (2149-2130) | SEQ ID NO 10 |
| Product 6F (2051-2070) | SEQ ID NO 11 |
| Product 6R (2556-2537) | SEQ ID NO 12 |

F= forward, R= reverse

Dye Primer Sequencing

Dye-primer sequencing using M13 forward and reverse primers was as described in the ABI protocol P/N 402114 for the ABI Prism™ dye primer cycle sequencing core kit with "AmpliTaq FS" DNA polymerase, modified in that the annealing temperature was 45 °C and DMSO was added to the cycle sequencing mix to a final concentration of 5%.

The extension reactions for each base were pooled, ethanol/sodium acetate precipitated, washed and resuspended in formamide loading buffer.

4.25% Acrylamide gels were run on an automated sequencer (ABI 377, Applied Biosystems).

## CLAIMS

1 A method for the diagnosis of a single nucleotide polymorphism in MCT-1 in a human, which method comprises determining the sequence of the nucleic acid of the human at at least one polymorphic position selected from one or more of the following positions: positions 1450, 1482 and 2461 in the polynucleotide sequence of the MCT-1 gene as defined by the position in SEQ ID NO: 13;

and determining the status of the human by reference to polymorphism in the MCT-1 gene.

2 A method accordning to claim one in which the single nucleotide polymorphismsare further defined as follows:

at position 1450 is presence of A and/or G;

at position 1482 is presence of A and/or T; and

at position 2461 is the presence of A and/or G.

3 A method for diagnosis according to claim 1 or 2 in which the sequence is determined by a method selected from amplification refractory mutation system and restriction fragment length polymorphism.

4 A polynucleotide comprising at least 20 bases of the human MCT-1 gene and comprising an allelic variant selected from any one of the following:

| variant | Position in SEQ ID NO 13 |
|---|---|
| G | 1450 |
| T | 1482 |
| G | 2461 |

5 An allele specific primer capable of detecting a MCT-1 gene polymorphism at one or more of the positions as defined in claim 1.

6 An allele-specific oligonucleotide probe capable of detecting a MCT-1 gene polymorphism at one or more of the positions defined in claim 1.

7 A diagnostic kit comprising an allele specific oligonucleotide probe as defined in claim 6 and/or an allele-specific primer as defined in claim 5.

8 Use of a single nucleotide polymorphism as defined in claim 1 as a genetic marker in linkage studies.

# ABSTRACT

## TITLE: CHEMICAL COMPOUNDS

This invention relates to polymorphisms in the human MCT-1 gene and corresponding novel allelic polypeptides encoded thereby. The invention also relates to methods and materials for analysing allelic variation in the MCT-1 gene, and to the use of MCT-1 polymorphism in treatment of diseases with MCT-1 transportable drugs.

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Leu Phe Thr His Arg Gly Phe Leu Leu Tyr Leu Ser Gly Asn Val Ile
260 265 270

Met Phe Phe Gly Leu Phe Ala Pro Leu Val Phe Leu Ser Ser Tyr Gly
275 280 285

Lys Ser Gln His Tyr Ser Ser Glu Lys Ser Ala Phe Leu Leu Ser Ile
290 295 300

Leu Ala Phe Val Asp Met Val Ala Arg Pro Ser Met Gly Leu Val Ala
305 310 315 320

Asn Thr Lys Pro Ile Arg Pro Arg Ile Gln Tyr Phe Phe Ala Ala Ser
325 330 335

Val Val Ala Asn Gly Val Cys His Met Leu Ala Pro Leu Ser Thr Thr
340 345 350

Tyr Val Gly Phe Cys Val Tyr Ala Gly Phe Phe Gly Phe Ala Phe Gly
355 360 365

Trp Leu Ser Ser Val Leu Phe Glu Thr Leu Met Asp Leu Val Gly Pro
370 375 380

Gln Arg Phe Ser Ser Ala Val Gly Leu Val Thr Ile Val Glu Cys Cys
385 390 395 400

Pro Val Leu Leu Gly Pro Pro Leu Leu Gly Arg Leu Asn Asp Met Tyr
405 410 415

Gly Asp Tyr Lys Tyr Thr Tyr Trp Ala Cys Gly Val Val Leu Ile Ile
420 425 430

Ser Gly Ile Tyr Leu Phe Ile Gly Met Gly Ile Asn Tyr Arg Leu Leu
435 440 445

Ala Lys Glu Gln Lys Ala Asn Glu Gln Lys Lys Glu Ser Lys Glu Glu
450 455 460

Glu Thr Ser Ile Asp Val Ala Gly Lys Pro Asn Glu Val Thr Lys Thr
465 470 475 480

Ala Glu Ser Pro Asp Gln Lys Asp Thr Glu Gly Gly Pro Lys Glu Glu
485 490 495

Glu Ser Pro Val
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